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# Leaving no stone unturned: three additional new species of *Atractus* ground snakes (Serpentes, Colubridae) from Ecuador discovered using a biogeographical approach

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## Abstract

The genus *Atractus* includes 146 species of cryptozoic snakes occurring from Panama to northeastern Argentina. Here, a molecular phylogeny of this genus is presented, which encompasses 29% (= 42; six are included here for the first time) of the species currently recognized. Morphological and phylogenetic support is found for three new species of ground snakes, which are described here based on their unique combination of molecular, meristic, and color pattern characteristics. The name *A. arangoi* Prado, 1939 is revalidated for a Colombian snake species previously subsumed under *A. major* Boulenger, 1894 based on new material collected in Ecuador. Reidentifications are provided for *Atractus* voucher specimens and sequences deposited in GenBank. With these changes, the number of *Atractus* reported in Ecuador increases from 27 to 31 species. Finally, attention is given to the importance of using a biogeographical framework that includes molecular data and a comprehensive geographic sampling when proposing species limits in complex taxonomic groups.

## Keywords

Biodiversity, biogeography, Colubridae, fossorial, phylogeny, new species, taxonomy

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## Introduction

*Atractus* Wagler, 1828 is the most speciose snake genus in the world (Uetz et al. 2022). There are 146 known species, and these numbers are likely to rise with the exploration of remote mountain ranges, the use of molecular tools in *Atractus* systematics, and the application of a biogeographical framework when establishing limits between species.

In Ecuador, the exploration of remote mountain ranges (e.g., the Cordillera de Guacamayos, Sumaco Volcano, and the Cordillera del Cóndor) within the last two decades has resulted in the discovery of at least six species of *Atractus*, including the most heavy-bodied and strikingly colored in the genus (Myers and Schargel 2006; Schargel et al. 2013; Arteaga et al. 2017; Passos et al. 2018; Melo-Sampaio et al. 2021). Unlike other snake genera inhabiting the same mountain ranges (e.g., *Dipsas*; see Arteaga et al. 2018), snakes in the genus *Atractus* inhabiting remote cloud forests and inter-Andean valleys are generally considered rare. Some are known only from their type localities (e.g., *A. cerberus* Arteaga et al., 2017) whereas for some species the males (e.g., *A. atlas* Passos et al., 2018) or juveniles (e.g., *A. touzeti* Schargel et al., 2013) have not yet been reported. All of this suggests that *Atractus* in general, with the exception of some locally abundant species (e.g., *A. marthae* Meneses-Pelayo & Passos, 2019), are difficult to find. Thus, species inhabiting poorly visited areas may remain undetected without long-term projects focused on cryptozoic herpetofauna (Myers 2003).

The use of molecular tools in *Atractus* systematics is also likely to increase the rate at which new species in this genus are detected and described. Only seven species of *Atractus* have been described using molecular data in addition to meristic and color pattern characteristics (Arteaga et al. 2017; Melo-Sampaio et al. 2019; Melo-Sampaio et al. 2021). Some of these new species were previously considered to be widespread, polychromatic, and difficult to diagnose (Savage 1960). Therefore, they probably would have never been detected using meristics and other morphological data alone. Furthermore, only approximately 30% of the current known diversity of the genus has been included in published phylogenetic analyses (i.e., Arteaga et al. 2017; Passos et al. 2022), and even a smaller percentage of the included species have been thoroughly sampled throughout their range. This lack of information presents both a challenge and an opportunity to uncover further cryptic diversity within the genus.

Finally, a mention should be made about the importance of using a biogeographical framework that includes molecular data and species distribution models (when the number and quality of locality records is sufficient for these analyses; see van Proosdij et al. 2015) when defining species limits within *Atractus*. Finding ground snakes along the Andes has showed us (Arteaga et al. 2013, 2017) and other authors (Savage 1955, 1960; Cisneros-Heredia 2005; Salazar-Valenzuela et al. 2014) that snakes in this genus have lower dispersal capacity than other colubrids and many species are endemic to a single mountain range or restricted to an isolated inter-Andean valley. Thus, the presence of the same *Atractus* species in two geographically isolated areas that are climatically and floristically distinct and are separated from each other by tens or even hundreds of kilometers of discontinuous habitat is unlikely. An example of this scenario

is *A. gigas* Myers & Schargel, 2006, a species previously considered to be endemic to the Pacific slopes of the Andes in Ecuador (Myers and Schargel 2006; Tolhurst et al. 2010; Arteaga et al. 2013), but later reported on the Amazonian slopes of the Andes in Peru (Passos et al. 2010). Although specimens from both localities may resemble each other in lepidosis, they differ in coloration, ecological requirements, and phylogenetic affinities. More recently, without explanation, but probably based on similarities in meristics, Passos et al. (2022) proposed the reidentification of 15 specimens of *Atractus* having sequences deposited in GenBank. Given that some of these reidentifications involve type series and the majority of them were done without providing an explanation, their validity is evaluated in this work.

To help clear the waters of *Atractus* taxonomy, in this work we present a curated phylogeny of the genus, reidentify *Atractus* sequences in GenBank, present the description of three new species, and provide the revalidation of a taxon previously subsumed under *A. major*.

## Materials and methods

### Ethics statement

This study was carried out in strict accordance with the guidelines for use of live amphibians and reptiles in field research (Beaupre et al. 2004) compiled by the American Society of Ichthyologists and Herpetologists (**ASIH**), the Herpetologists' League (**HL**) and the Society for the Study of Amphibians and Reptiles (**SSAR**). All procedures with animals (see below) were reviewed by the Ministerio del Ambiente, Agua y Transición Ecológica (**MAATE**) and specifically approved as part of obtaining the following field permits for research and collection: MAE-DNB-CM-2015-0017 (granted to Universidad Tecnológica Indoamérica), MAE-DNB-CM-2018-0105 and MAATE-DBI-CM-2022-0245 (granted to Universidad San Francisco de Quito), and 004-AIC-DPC-B-MAE-18 (granted to Universidad del Azuay). Specimens were euthanized with 20% benzocaine, fixed in 10% formalin or 90% ethanol, and stored in 70% ethanol. Museum vouchers were deposited at Museo de Zoología de la Universidad Tecnológica Indoamérica (**MZUTI**), Museo de Zoología de la Universidad San Francisco de Quito (**ZSFQ**), Museo de Zoología de la Universidad del Azuay (**MZUA**), and the herpetology collection at Bioparque Amaru (**AMARU**). Specimens labeled JMG were also deposited at ZSFQ.

### Common names

Criteria for common name designation are as proposed by Caramaschi et al. (2006) and Coloma and Guayasamin (2011–2017), reviewed by Arteaga et al. (2019). These are as follows (in order of importance): (i) the etymological intention (implicit or explicit) that the authors used when naming the species (specific epithet); (ii) a common

name that is already widely used in the scientific literature; (iii) a common name that has an important ancestral or cultural meaning; (iv) a common name based on any distinctive aspect of the species (distribution, morphology, behavior, etc.).

## Morphological data

Our terminology for *Atractus* cephalic shields follows Savage (1960), diagnoses and descriptions generally follow Passos et al. (2009a), and ventral and subcaudal counts follow Dowling (1951). We examined comparative alcohol-preserved specimens from the herpetology collections at MZUTI, MZUA, ZSFQ, American Museum of Natural History (**AMNH**), Museo de Zoología de la Pontificia Universidad Católica del Ecuador (**QCAZ**), and Muséum National d'Histoire Naturelle (**MNHN**) (Table 1). Morphological measurements were taken with measuring tapes to the nearest 1 mm, or with digital calipers to the nearest 0.1 mm. Abbreviations are as follows: snout-vent length (**SVL**); tail length (**TL**). Sex was determined by establishing the presence/absence of hemipenes through a subcaudal incision at the base of the tail unless hemipenes were everted.

## Sampling

Tissue samples from 12 individuals representing seven species (including the three new species described here) were obtained in Ecuador. All specimens included in the genetic analyses were morphologically identified according to Savage (1960), Arteaga et al. (2017), Melo-Sampaio et al. (2021), and Arteaga et al. (2022). We generated sequence data for samples marked with an asterisk under Appendix I, which includes museum vouchers at MZUTI, MZUA, and ZSFQ.

## Laboratory techniques

Genomic DNA was extracted from 96% ethanol-preserved tissue samples (liver, muscle tissue, or scales) using either a guanidinium isothiocyanate extraction protocol (Peñafiel et al. 2020), or a modified salt precipitation method based on the Puregene DNA purification kit (Gentra Systems). The nucleotide sequences of the primers and the PCR conditions applied to each primer pair are detailed in Appendix II. PCR products were cleaned with either ExoSAP-IT (Affymetrix, Cleveland, OH), or Exonuclease I and Alkaline Phosphatase (Illustra ExoProStar by GE Healthcare) before they were sent to Macrogen Inc (Seoul, South Korea) for sequencing. All PCR products were sequenced in both forward and reverse directions with the same primers that were used for amplification. The edited sequences were deposited in GenBank (Appendix I).

## DNA phylogenetic analyses

A total of 274 DNA sequences were used to build a phylogenetic tree of the genus *Atractus*, of which 32 were generated during this work and 242 were downloaded from GenBank, most of which were produced by Arteaga et al. (2017), Melo-Sampaio et al. (2021), and

**Table 1.** Locality data for specimens examined in this study. Coordinates represent actual GPS readings taken at the locality of collection or georeferencing attempts from gazetteers under standard guidelines, although some variation from the exact collecting locality will be present. Similarly, elevations are taken from Google Earth and may not exactly match the elevations as originally reported.

| Species | Voucher | Country | Province | Locality | Latitude, Longitude | Elev. (m) |
|---|---|---|---|---|---|---|
| *A. arangoi* | **DHMECN 8343** | Ecuador | Sucumbíos | Bloque 27 | 0.32271, -76.19300 | 264 |
| *A. arangoi* | **ZSFQ 4947** | Ecuador | Napo | Jatun Sacha Biological Station | -1.06633, -77.61640 | 423 |
| *A. arangoi* | **ZSFQ 4948** | Ecuador | Napo | Jatun Sacha Biological Station | -1.06633, -77.61640 | 423 |
| *A. discovery* sp. nov. | **MZUA.RE.0466** | Ecuador | Morona Santiago | Campamento Arenales | -2.59253, -78.56507 | 2057 |
| *A. discovery* sp. nov. | **ZSFQ 4936** | Ecuador | Azuay | Amaluza | -2.61583, -78.56538 | 2002 |
| *A. discovery* sp. nov. | **ZSFQ 4937** | Ecuador | Azuay | Amaluza | -2.61583, -78.56538 | 2002 |
| *A. major* | **MNHN 0.6149** | Ecuador | – | – | – | – |
| *A. major* | **QCAZ 11565** | Ecuador | Orellana | Tambococha | -0.97839, -75.42569 | 194 |
| *A. major* | **QCAZ 11587** | Ecuador | Orellana | Tambococha | -1.03981, -75.44849 | 210 |
| *A. major* | **QCAZ 11596** | Ecuador | Orellana | Tambococha | -0.97839, -75.42569 | 194 |
| *A. major* | **QCAZ 11809** | Ecuador | Pastaza | Campo Villano B | -1.45745, -77.44455 | 331 |
| *A. major* | **QCAZ 4691** | Ecuador | Pastaza | Río Sarayakillo | -1.72754, -77.48048 | 434 |
| *A. major* | **QCAZ 4895** | Ecuador | Orellana | Vía Pompeya Sur-Iro | -0.99307, -76.24904 | 246 |
| *A. major* | **QCAZ 7881** | Ecuador | Sucumbíos | Pañacocha | -0.44791, -76.07097 | 240 |
| *A. major* | **QCAZ 7896** | Ecuador | Orellana | Vía Pompeya Sur-Iro | -0.99320, -76.24907 | 246 |
| *A. major* | **QCAZ 8040** | Ecuador | Napo | Comunidad Gareno | -1.04856, -77.37742 | 334 |
| *A. major* | **QCAZR 11744** | Ecuador | Pastaza | Lorocachi | -1.65567, -75.96886 | 212 |
| *A. major* | **ZSFQ 4955** | Ecuador | Morona Santiago | Macas-Riobamba | -2.25674, -78.16797 | 1148 |
| *A. michaelsabini* sp. nov. | **AMNH 18325** | Ecuador | El Oro | El Chiral | -3.63825, -79.59723 | 1841 |
| *A. michaelsabini* sp. nov. | **AMNH 22110** | Ecuador | El Oro | La Chonta | -3.56585, -79.85144 | 1025 |
| *A. michaelsabini* sp. nov. | **AMNH 22111** | Ecuador | El Oro | La Chonta | -3.56585, -79.85144 | 1025 |
| *A. michaelsabini* sp. nov. | **DHMECN 7644** | Ecuador | Azuay | Reserva Yunguilla | -3.22684, -79.27520 | 1748 |
| *A. michaelsabini* sp. nov. | **DHMECN 7645** | Ecuador | Azuay | Reserva Yunguilla | -3.22684, -79.27520 | 1748 |
| *A. michaelsabini* sp. nov. | **QCAZ 7887** | Ecuador | El Oro | Guanazán | -3.44139, -79.49417 | 2596 |
| *A. michaelsabini* sp. nov. | **QCAZ 7902** | Ecuador | El Oro | Guanazán | -3.44668, -79.49051 | 2663 |
| *A. michaelsabini* sp. nov. | **QCAZ 9643** | Ecuador | El Oro | El Panecillo | -3.46753, -79.48248 | 2775 |
| *A. michaelsabini* sp. nov. | **QCAZ 9652** | Ecuador | El Oro | El Panecillo | -3.46753, -79.48248 | 2775 |
| *A. michaelsabini* sp. nov. | **ZSFQ 4938** | Ecuador | Azuay | Corraleja | -3.38740, -79.22785 | 2660 |
| *A. michaelsabini* sp. nov. | **ZSFQ 4939** | Ecuador | El Oro | Guanazán | -3.46753, -79.48248 | 2750 |
| *A. pachacamac* | **ZSFQ 4954** | Ecuador | Morona Santiago | Macas-Riobamba | -2.24087, -78.27632 | 1644 |
| *A. resplendens* | **ZSFQ 4953** | Ecuador | Tungurahua | Montañas de San Antonio | -1.43413, -78.40726 | 2655 |
| *A. resplendens* | **ZSFQ 4952** | Ecuador | Tungurahua | Montañas de San Antonio | -1.43413, -78.40726 | 2655 |
| *A. resplendens* | **ZSFQ 4951** | Ecuador | Tungurahua | Montañas de San Antonio | -1.43413, -78.40726 | 2655 |
| *A. roulei* | **MNHN 1906.0243** | Ecuador | Chimborazo | Alausí | -2.20636, -78.84611 | 2400 |
| *A. roulei* | **MZUA.RE.0080** | Ecuador | Azuay | Miguir, 10 km E of | -2.78771, -79.37132 | 2596 |
| *A. roulei* | **MZUTI 5107** | Ecuador | Bolívar | Above Balzapamba | -1.83601, -79.13322 | 2026 |
| *A. roulei* | **QCAZ 6256** | Ecuador | Azuay | Hierba Mala | -2.70430, -79.43367 | 2427 |
| *A. roulei* | **ZSFQ 4943** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. roulei* | **ZSFQ 4944** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. roulei* | **ZSFQ 4942** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. roulei* | **ZSFQ 4941** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. roulei* | **ZSFQ 4940** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. roulei* | **ZSFQ 4945** | Ecuador | Chimborazo | Tixán | -2.16174, -78.81227 | 2892 |
| *A. zgap* sp. nov. | **ZSFQ 4946** | Ecuador | Napo | Santa Rosa | -0.31004, -77.78591 | 1500 |
| *A. zgap* sp. nov. | **QCAZ 12666** | Ecuador | Napo | Borja, 1 km NE of | -0.40954, -77.84005 | 1703 |
| *A. zgap* sp. nov. | **QCAZ 5183** | Ecuador | Napo | Bosque La Cascada | -0.14572, -77.49593 | 1460 |

Passos et al. (2022). Of these, 85 sequences are 367–516 bp long fragments of the 16S gene, 66 are 578–1,079 bp long fragments of the CYTB gene, 69 are 567–849 bp long fragments of the ND4 gene, 18 are 513–573 bp long fragments of the C-MOS gene, 19 are 386–516 bp long fragments of the NT3 gene, and 17 are 736 bp long fragments of the RAG-1 gene. New sequences were edited and assembled using the program Geneious ProTM 2021.1.1 (Drummond et al. 2021) and aligned with those downloaded from GenBank (Appendix I) using MAFFT v.7 (Katoh and Standley 2013) under the default parameters in Geneious ProTM 2021.1.1. Genes were combined into a single matrix with 16 partitions, one per non-coding gene and three per protein coding gene corresponding to each codon position. The best partition strategies along with the best-fit models of evolution were obtained in PartitionFinder 2.1.1 (Lanfear et al. 2016) under the Bayesian information criterion.

Phylogenetic relationships were assessed under both a Bayesian inference (**BI**) approach in MrBayes 3.2.0 (Ronquist and Huelsenbeck 2013) and a maximum likelihood (**ML**) approach in RAxML-NG v. 1.1.0 (Kozlov et al. 2019). For the ML analysis, nodal support was assessed using the *standard* bootstrapping algorithm with 1000 non-parametric bootstraps. For the BI analysis, four independent analyses were performed to reduce the chance of converging on a local optimum. Each analysis consisted of 6,666,667 generations and four Markov chains with default heating settings. Trees were sampled every 1,000 generations and 25% of them were arbitrarily discarded as "burn-in." The resulting 5,000 saved trees per analysis were used to calculate posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree. We used Tracer 1.7.2 (Rambaut et al. 2022) to assess convergence and effective sample sizes (ESS) for all parameters. Additionally, we verified that the average standard deviation of split frequencies between chains and the potential scale reduction factor (**PSRF**) of all the estimated parameters approached values of ≤ 0.01 and 1, respectively. Genetic distances between *Atractus roulei* Despax, 1910 and its sister species were calculated using the uncorrected distance matrix in Geneious ProTM 2021.1.1. GenBank accession numbers are listed in Appendix I.

## Distribution maps and ecological niche models

We present ranges of occurrence for five species of *Atractus*, including the three new species described here. Presence localities are derived from museum vouchers (Table 1), photographic records (iNaturalist), and the literature (all summarized under Suppl. material 1: Table S1). For three of the five species, a binary environmental niche model (ENM) accompanies the dot maps. These models estimate potential areas of distribution on the basis of observed presences and a set of environmental predictors (Elith and Leathwick 2009). To delimit the occupancy areas and the potential species distribution, we used the BAM diagram proposal (Soberón and Peterson 2005; Peterson et al. 2011). To create the models, we used presence localities listed under Suppl. material 1: Table S1, 19 bioclimatic variables from Worldclim 1.4 (Hijmans et al. 2005), and Maxent 3.4.1k, an algorithm based on the principle of maximum entropy (Phillips et al. 2006; Elith et al. 2011; Renner and Warton 2013).

For the first explorative exercise, we used the 19 climate layers from the WorldClim project and assessed which variables were the most important for the model,

according to the Jackknife test calculated in MaxEnt (Royle et al. 2012). Correlated environmental variables ($r < 0.8$) were identified using the PEARSON correlation test of PAST 3. In a second modelling exercise, we used the locality records for each species (Suppl. material 1: Table S1) and the variables identified in the first approach to generate the species distribution. 5,000 iterations were specified to the program with clamping and no extrapolation. All other parameters in MaxEnt were maintained at default settings. To create the binary environmental niche models, suitable areas were distinguished from unsuitable areas by setting a *minimum training presence* threshold value. The logistic format was used to obtain the values for habitat suitability (continuous probability from 0 to 1), which were subsequently converted to binary presence-absence values on the basis of the established threshold value, defined herein as *the minimum training presence*. The convergence threshold was set to $10^{-5}$, maximum iterations to 500, and the regularization parameter to "auto".

## Results

### Molecular phylogeny and taxonomic consequences

Selected partitions and models of evolution are presented in Table 2. We consider strong support for a clade when Bayesian analyses yield posterior probability values > 95%, following Felsenstein (2004), or when bootstrap values are greater than 70%. The overall topology and support of the BI (Fig. 1) and ML (Suppl. material 2: Figure S1) analyses are similar to that of Arteaga et al. (2017) and Passos et al. (2022). Species of the *Atractus roulei* species group are sister to all other sampled *Atractus* in the BI analysis, a view contrary to the ML analysis and to Murphy et al. (2019), in which *A. trilineatus* Wagler, 1928 and *A. boimirim* Passos et al., 2016, respectively are recovered as sister to all other *Atractus*. Below, we outline some differences between our analysis and those published in Murphy et al. (2019) and Passos et al. (2022).

*Atractus roulei* is the strongly supported sister species of *A. carrioni* Parker, 1930, a relationship recovered in previous studies, but we found additional geographically structured genetic divergence within the former species (Figs 1, 2). We found moderate support for the placement of *A. trilineatus* as sister to *A. major* sensu Schargel et al. (2013), but strong support for the reciprocal monophyly between snakes assignable to *A. arangoi*, previously subsumed under *A. major*, and all other samples of *A. major*, including samples from throughout the species' area of distribution. Samples labeled *A. arangoi* in our phylogeny are not closely related to *A. torquatus* (Duméril, Bibron, & Duméril, 1854), a name that has been applied to Ecuadorian specimens of the former (see Maynard et al. 2017). Our sample of *A. touzeti* Schargel et al., 2013 from the type locality is strongly supported as sister to the sample of *A. atlas* Passos et al., 2018. We found strong support for the relationship between *A. resplendens* Werner, 1901 from near the type locality and a new species from southeastern Ecuador. Our included samples of *A. orcesi* Savage, 1955 form a strongly supported sister clade to *A. duboisi* (Boulenger,

**Table 2.** Partition scheme and models of evolution used in phylogenetic analyses. Numbers in parentheses indicate codon position.

| Partition | Best model | Gene regions | Number of aligned sites |
|---|---|---|---|
| 1 | GTR+I+G | 16S, cytb(3), ND4(1), NT3(1) | 1202 |
| 2 | HKY+I+G | cytb(1), ND4(2) | 631 |
| 3 | GTR+I+G | cytb(2), ND4(3) | 630 |
| 4 | JC | CMOS(1), NT3(3) | 305 |
| 5 | K80+I | CMOS(2), NT3(2), RAG1(2), RAG1(3) | 794 |
| 6 | HKY | CMOS(3), RAG1(1) | 423 |

1880). A new species previously confused with *A. ecuadorensis* Savage, 1955, *A. orcesi*, and *A. resplendens* is not closely related to any of these species, but is recovered as the strongly supported sister species to a clade that contains *A. ukupacha* Melo-Sampaio et al., 2021, *A. pachacamac* Melo-Sampaio et al., 2021, *A. snethlageae* da Cunha & do Nascimento, 1983, *A. dapsilis* Melo-Sampaio et al., 2019, *A. schach* (Boie, 1827), and *A. trefauti* Melo-Sampaio et al., 2019. The latter two are sister species and their topological distance is smaller than intraspecific distances in other *Atractus* species sampled.

We find strong support for the relationship between members of the *Atractus iridescens* species group, which mirrors the results of Arteaga et al. (2017) and Murphy et al. (2019), and even those of Passos et al. (2022), although in the latter work some the terminals have been renamed. However, in the ML analysis (Suppl. material 2: Figure S1), *A. dunni* Savage, 1955 is weakly nested within *A. microrhynchus* Cope, 1868. Finally, we excluded *A. imperfectus* Myers, 2003 (voucher CH 9399) from the analyses as the short sequence available for comparison in GenBank (gene fragment 16S) represented a rogue taxon that assumed varying phylogenetic positions in the tree collection used to build the consensus tree.

## Systematic accounts

We name or provide redescriptions only for species that are monophyletic in our molecular phylogeny and share diagnostic features of their coloration pattern and lepidosis. Based on these species' delimitation criteria, which follow the general species concept of de Queiroz (2007), we describe three new species of *Atractus*.

***Atractus discovery* sp. nov.**
https://zoobank.org/0343A95C-BC4B-4654-8333-55D8A34CD2EF
Figs 3, 4, 5d
Proposed standard english name: Discovery Ground Snake.
Proposed standard spanish name: Culebra tierrera de Discovery.

**Holotype.** ZSFQ 4937 (Figs 3, 4), adult male collected by Alejandro Arteaga and Amanda Quezada at Amaluza, Azuay province, Ecuador (S2.61582, W78.56537; 2002 m).

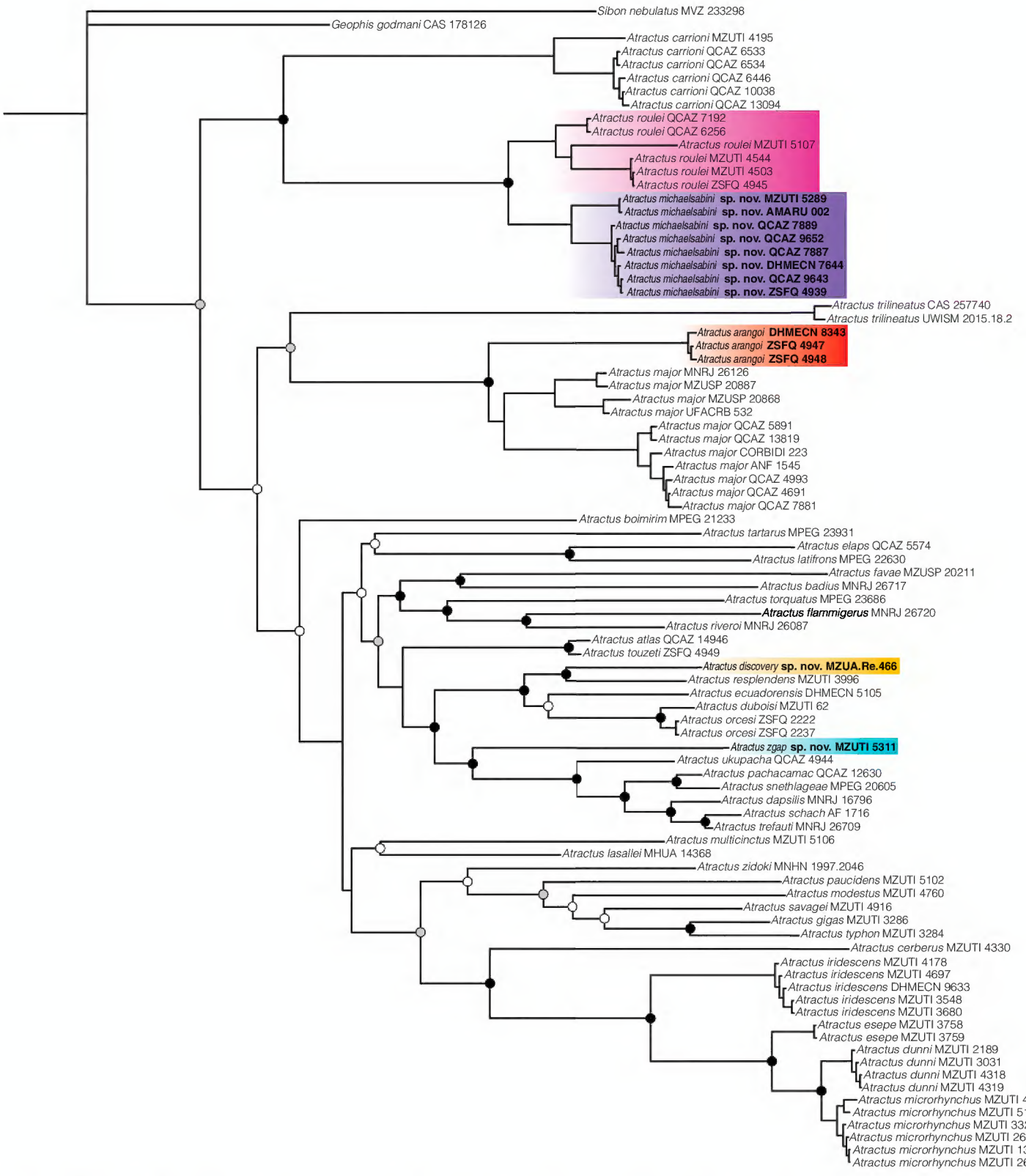
Sibon nebulatus MVZ 233298
Geophis godmani CAS 178126
Atractus carrioni MZUTI 4195
Atractus carrioni QCAZ 6533
Atractus carrioni QCAZ 6534
Atractus carrioni QCAZ 6446
Atractus carrioni QCAZ 10038
Atractus carrioni QCAZ 13094
Atractus roulei QCAZ 7192
Atractus roulei QCAZ 6256
Atractus roulei MZUTI 5107
Atractus roulei MZUTI 4544
Atractus roulei MZUTI 4503
Atractus roulei ZSFQ 4945
Atractus michaelsabini sp. nov. MZUTI 5289
Atractus michaelsabini sp. nov. AMARU 002
Atractus michaelsabini sp. nov. QCAZ 7889
Atractus michaelsabini sp. nov. QCAZ 9652
Atractus michaelsabini sp. nov. QCAZ 7887
Atractus michaelsabini sp. nov. DHMECN 7644
Atractus michaelsabini sp. nov. QCAZ 9643
Atractus michaelsabini sp. nov. ZSFQ 4939
Atractus trilineatus CAS 257740
Atractus trilineatus UWISM 2015.18.2
Atractus arangoi DHMECN 8343
Atractus arangoi ZSFQ 4947
Atractus arangoi ZSFQ 4948
Atractus major MNRJ 26126
Atractus major MZUSP 20887
Atractus major MZUSP 20868
Atractus major UFACRB 532
Atractus major QCAZ 5891
Atractus major QCAZ 13819
Atractus major CORBIDI 223
Atractus major ANF 1545
Atractus major QCAZ 4993
Atractus major QCAZ 4691
Atractus major QCAZ 7881
Atractus boimirim MPEG 21233
Atractus tartarus MPEG 23931
Atractus elaps QCAZ 5574
Atractus latifrons MPEG 22630
Atractus favae MZUSP 20211
Atractus badius MNRJ 26717
Atractus torquatus MPEG 23686
Atractus flammigerus MNRJ 26720
Atractus riveroi MNRJ 26087
Atractus atlas QCAZ 14946
Atractus touzeti ZSFQ 4949
Atractus discovery sp. nov. MZUA.Re.466
Atractus resplendens MZUTI 3996
Atractus ecuadorensis DHMECN 5105
Atractus duboisi MZUTI 62
Atractus orcesi ZSFQ 2222
Atractus orcesi ZSFQ 2237
Atractus zgap sp. nov. MZUTI 5311
Atractus ukupacha QCAZ 4944
Atractus pachacamac QCAZ 12630
Atractus snethlageae MPEG 20605
Atractus dapsilis MNRJ 16796
Atractus schach AF 1716
Atractus trefauti MNRJ 26709
Atractus multicinctus MZUTI 5106
Atractus lasallei MHUA 14368
Atractus zidoki MNHN 1997.2046
Atractus paucidens MZUTI 5102
Atractus modestus MZUTI 4760
Atractus savagei MZUTI 4916
Atractus gigas MZUTI 3286
Atractus typhon MZUTI 3284
Atractus cerberus MZUTI 4330
Atractus iridescens MZUTI 4178
Atractus iridescens MZUTI 4697
Atractus iridescens DHMECN 9633
Atractus iridescens MZUTI 3548
Atractus iridescens MZUTI 3680
Atractus esepe MZUTI 3758
Atractus esepe MZUTI 3759
Atractus dunni MZUTI 2189
Atractus dunni MZUTI 3031
Atractus dunni MZUTI 4318
Atractus dunni MZUTI 4319
Atractus microrhynchus MZUTI 4122
Atractus microrhynchus MZUTI 5109
Atractus microrhynchus MZUTI 3323
Atractus microrhynchus MZUTI 2649
Atractus microrhynchus MZUTI 1385
Atractus microrhynchus MZUTI 2650

**Figure 1.** Phylogenetic relationships within *Atractus* inferred using a Bayesian inference and derived from analysis of 3,985 bp of DNA (gene fragments 16S, CYTB, ND4, C-MOS, NT3, and RAG1). Support values on intra-specific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal. Black dots indicate clades with posterior probability values from 95–100%. Grey dots indicate values from 70–94%. White dots indicate values from 50–69% (values < 50% not shown). Colored clades correspond to the species' distribution presented in the map of Fig. 2. New or resurrected species are indicated in bold type.

**Paratypes.** ZSFQ 4936 (Fig 5d), adult female collected by Alejandro Arteaga and Amanda Quezada at the type locality. MZUA.Re.466, adult female collected on 16 November 2018 at Campamento Arenales, Morona Santiago province, Ecuador (S2.59253, W78.56507; 2057 m).

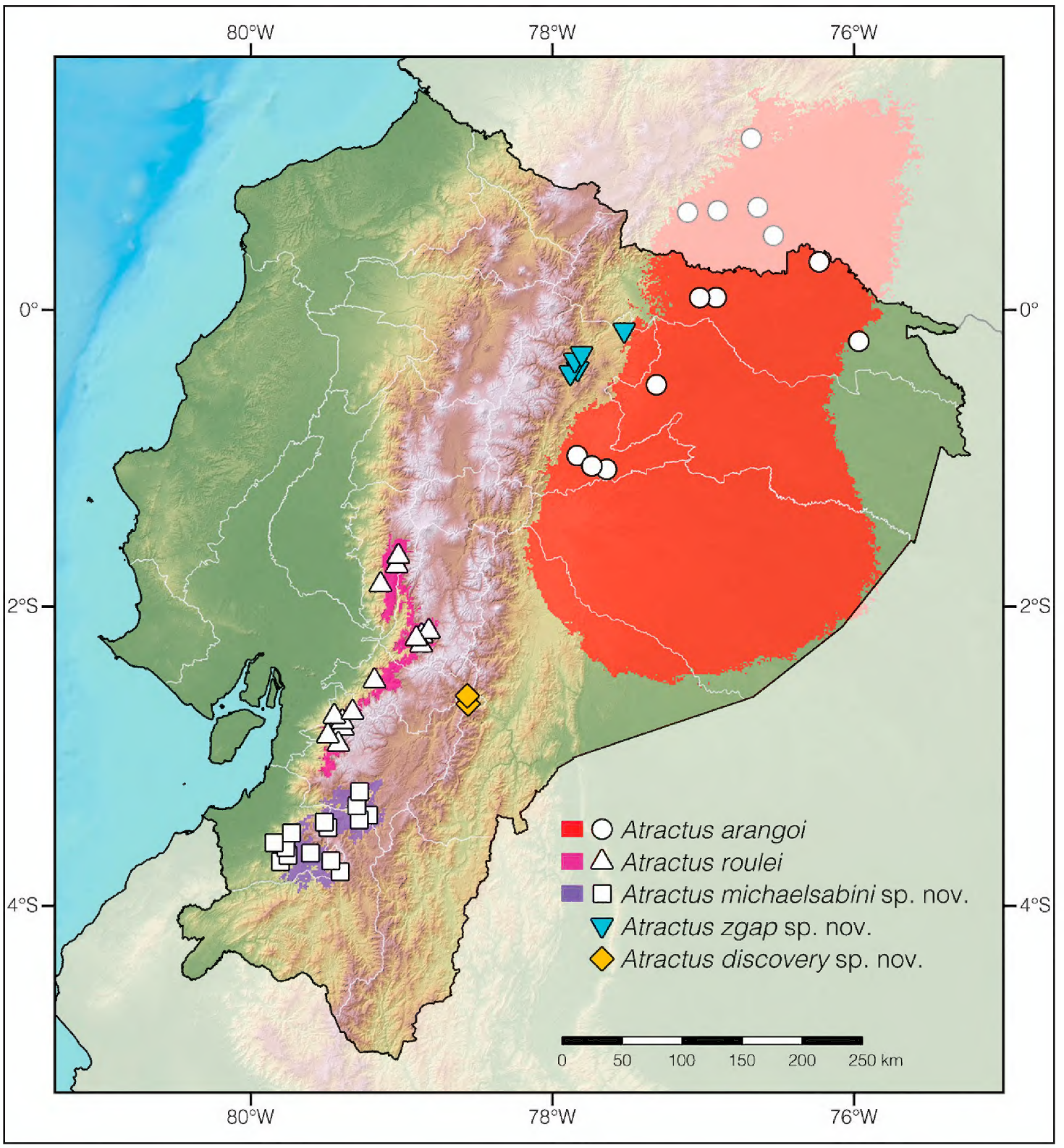


**Figure 2.** Distribution of *Atractus arangoi*, *A. roulei*, *A. michaelsabini* sp. nov., *A. zgap* sp. nov., and *A. discovery* sp. nov. in Ecuador and adjacent Colombia. White dots represent localities listed under Suppl. material 1. Each colored area is a geographic representation of the suitable environmental conditions for one of the clades recovered in the phylogeny of Fig. 1.

**Diagnosis.** *Atractus discovery* sp. nov. is placed in the genus *Atractus*, as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) one postocular; (3) loreal 2.5–3 × longer than high; (4) temporals 1+2; (5) eight supralabials, fourth and fifth contacting orbit; (6) seven infralabials, first four contacting chinshields; (7) six or seven maxillary teeth; (8) one row of gular scales; (9) three preventrals; (10) 168 ventrals in the male holotype (Fig. 3b) and 170–172 ventrals in females; (11) 27 subcaudals in the male holotype and 17–18 subcaudals in females;

(12) dorsal ground color light brown with faint stippling of a darker shade (Figs 3a, 5d); (13) venter yellow with a brown ventral stripe (Fig. 3b); (14) 284 mm SVL in the male holotype and 308–328 mm SVL in females; (15) 28 mm TL in the male holotype and 19–24 mm TL in females.

**Comparisons.** *Atractus discovery* sp. nov. differs from most of its congeners by having a bright yellow belly with a conspicuous dark brown longitudinal stripe. This species is compared to other small brownish congeneric ground snakes distributed along the Amazonian slopes of the Andes (most of these are pictured in Fig. 5): *Atractus avernus* Passos et al., 2009b, *A. duboisi*, *A. ecuadorensis*, *A. zgap* sp. nov., *A. occipitoalbus* (Jan, 1862), *A. orcesi*, and *A. resplendens*. From *A. avernus*, *A. duboisi*, *A. occipitoalbus*, and *A. orcesi*, the new species differs in having 17/17/17 (instead of 15/15/15) dorsal scale rows. From *A. ecuadorensis*, *A. zgap* sp. nov., and *A. resplendens*, it differs in having a bright yellow belly with a conspicuous dark brown longitudinal stripe. From *A. ecuadorensis* and *A. zgap* sp. nov., it further differs by having one (instead of two) postocular scale (Fig. 4c).

**Description of holotype.** Adult male, SVL 284 mm, tail length 28 mm (9.9% SVL); body diameter 7.8 mm; head length 8.8 mm (3.1% SVL); head width 5.6 mm (2.0% SVL); interocular distance 3.4 mm; head slightly distinct from body; snout-orbit distance 3.4 mm; rostral 1.6 mm wide, ca. as broad as high; internasals 0.9 mm wide; prefrontals 2.1 mm wide; frontal 2.9 mm wide, with a curvilinear triangular shape in dorsal view; parietals 2.2 mm wide, ~ 2 × as long as wide; nasal divided; loreal 2.0 mm long, ~ 3 × longer than high; eye diameter 1.1 mm; pupil round; supraoculars 1.3 mm wide; one postocular; temporals 1+2, upper posterior temporal elongate; eight supralabials, fourth and fifth contacting orbit; symphysial 1.0 mm wide, ~ 2 × as broad as long and separated from chinshields by first pair of infralabials; seven infralabials, first four contacting chinshields; chinshields ~ 2 × as long as broad, posterior chinshields absent; four rows of gular scales; dorsal scales arranged in 17/17/17 rows, smooth without apical pits; two preventrals; ventrals 168; anal plate single; 27 paired subcaudals.

**Natural history.** The three known specimens of *Atractus discovery* sp. nov. were found in open areas adjacent to cloud forest border. MZUA.Re.466 was crawling at ground level at around 7:30 pm. It was crossing a series of cement stairs. ZSFQ 4936 and ZSFQ 4937 were found during a cloudy day, buried 15–40 cm under soft soil at the border between the clearing of a graveyard, pastures, and remnants of native vegetation.

**Distribution.** *Atractus discovery* sp. nov. is known only from two localities (Arenales and Amaluza, listed under Suppl. material 1: Table S1) on each side of the Río Paute, in the Ecuadorian provinces Azuay and Morona Santiago, at elevations 2002–2057 m a.s.l. The airline distance between the two localities is 2.6 km (Fig. 2).

**Etymology.** The specific epithet *discovery* is used as a noun in apposition and honors 'The Explorers Club Discovery Expedition Grants' (https://www.explorers.org/grants) initiative, a program seeking to foster scientific understanding for the betterment of humanity and all life on Earth and beyond. The grant program supports researchers and explorers from around the world in their quest to mitigate climate change, prevent the extinction of species and cultures, and ensure the health of the Earth and its inhabitants. 'The Explorers Club Discovery Expedition Grants' program funded the expedition that resulted in the discovery of this new species of snake.

**Figure 3.** Adult male holotype of *Atractus discovery* sp. nov. ZSFQ 4937 in **a** dorsal and **b** ventral view.

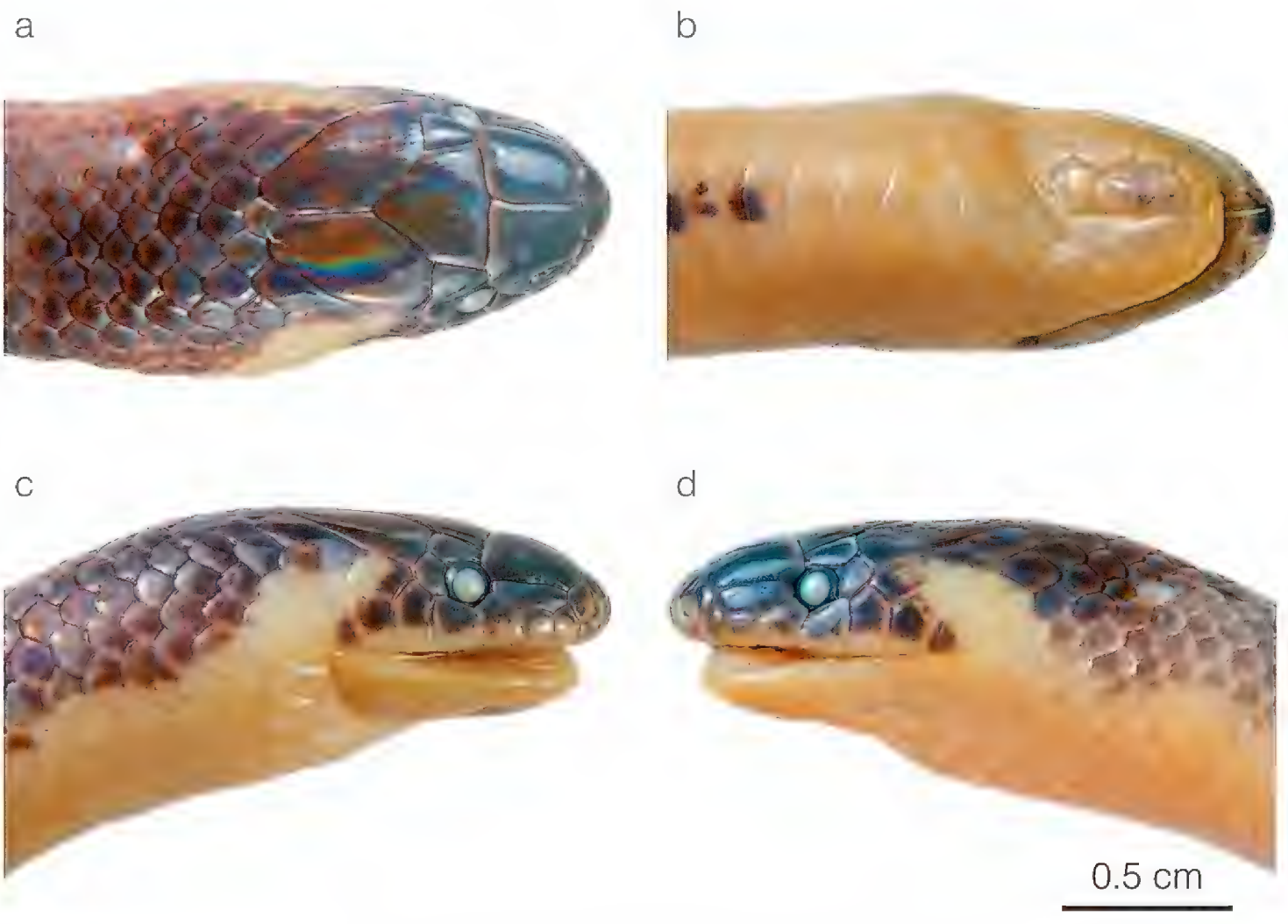


**Figure 4.** Head of the adult male holotype of *Atractus discovery* sp. nov. ZSFQ 4937 in **a** dorsal **b** ventral **c** lateral right, and **d** lateral left view.

**Figure 5.** Photographs of living specimens of brown-colored *Atractus* occurring along the Amazonian slopes of the Andes in Ecuador **a** *A. arangoi* ZSFQ 4948 from Jatun Sacha Biological Reserve, Napo province, Ecuador **b** *A. resplendens* ZSFQ 4953 from Montañas de San Antonio, Tungurahua province, Ecuador **c** *A. duboisi* from Orito Yacu, Napo province, Ecuador **d** *A. discovery* sp. nov. ZSFQ 4936 from Amaluza, Azuay province, Ecuador **e** *A. orcesi* ZSFQ 2234 from El Higuerón, Sucumbíos province, Ecuador **f** *A. pachacamac* from Nangaritza, Zamora Chinchipe province, Ecuador **g** *A. zgap* sp. nov. ZSFQ 4946 from Santa Rosa, Napo province, Ecuador **h** *A. occipitoalbus* JMG-2077 from Macas, Morona Santiago province, Ecuador **i** *A. major* from Jatun Sacha Biological Reserve, Napo province, Ecuador; and **j** *A. major* from Reserva Natural Palmarí, Amazonas state, Brazil (photo by Sebastián Di Doménico).

**Conservation status.** We consider *Atractus discovery* sp. nov. to be Data Deficient, following IUCN Red List criteria, because the species belongs to a poorly studied genus of snakes and is known only from three specimens collected recently in a single river valley (Río Paute) in the Amazonian slopes of the Ecuadorian Andes. In addition to the presence of a system of major hydroelectric dams in this valley, most of the native cloud forest habitat in the segment between Amaluza and Arenales has been converted to pastures. However, we consider there is insufficient data to estimate whether this new snake species is restricted to the immediate environs of the type locality or if it is widely distributed along the unexplored cloud forests of the adjacent Sangay National Park.

***Atractus zgap* sp. nov.**

https://zoobank.org/A9A58D40-CF58-4267-A691-B5E776B43C1B

Figs 5g, 6, 7

Proposed standard English name: ZGAP Ground Snake.

Proposed standard Spanish name: Culebra tierrera de ZGAP.

**Holotype.** ZSFQ 4946 (Figs 5g, 6, 7), adult female collected by Diego Piñán at Santa Rosa, Napo province, Ecuador (S0.31004, W77.78591; 1500 m).

**Paratypes.** MZUTI 5311, adult female collected by Diego Piñán in February 2017 at El Chaco, Napo Province, Ecuador (S0.31004, W77.78591; 1500 m). QCAZ 12666, a juvenile collected by Pablo Medrano on 16 May 2014 at San Francisco de Borja, Napo province, Ecuador (S0.40953, W77.84005; 1703 m). QCAZ 5183, a juvenile collected by Patricia Bejarano on 13 November 2011 at Bosque Protector "La Cascada," Napo province, Ecuador (S0.14572, W77.49593; 1460 m).

**Diagnosis.** *Atractus zgap* sp. nov. is placed in the genus *Atractus*, as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 17/17/17 smooth dorsals; (2) two postoculars; (3) loreal 2 × longer than high; (4) temporals 1+2; (5) seven supralabials, third and fourth contacting orbit; (6) seven infralabials, first three contacting chinshields; (7) seven maxillary teeth; (8) three rows of gular scales; (9) two or three preventrals; (10) 173–177 ventrals in females; (11) 31 subcaudals in an uncollected male and 25–27 subcaudals in females; (12) dorsal ground color brown with faint dark longitudinal lines (Figs 5g, 6a); (13) venter yellow with fine brown stippling (Fig. 6b); (14) 376 mm SVL in the female holotype; (15) 37 mm TL in the female holotype.

**Comparisons.** *Atractus zgap* sp. nov. is compared to other small brownish congeneric ground snakes distributed along the Amazonian slopes of the Andes (most of these are illustrated in Fig. 5): *Atractus avernus*, *A. duboisi*, *A. discovery* sp. nov., *A. ecuadorensis*, *A. occipitoalbus*, *A. orcesi*, and *A. resplendens*. From *A. avernus*, *A. duboisi*, *A. occipitoalbus*, and *A. orcesi*, the new species differs in having 17/17/17 dorsal scale rows. From *A. discovery* sp. nov., the new species differs in having two postocular scales (Fig. 7c) and no dark ventral stripe. From *A. ecuadorensis*, the new species differs in

**Figure 6.** Adult female holotype of *Atractus zgap* sp. nov. ZSFQ 4946 in **a** dorsal and **b** ventral view.

having fewer (31 instead of 41) subcaudals in males, seven (instead of five or six) infralabials, a shorter (2 × instead of 3 × longer than high) loreal, frontal longer than prefrontals, and five faint (instead of six or seven clearly defined) longitudinal black lines (Figs 5g, 6). From *A. resplendens*, the new species differs in having a shorter (2 × instead of 3 × longer than high) loreal, two (instead of one) postoculars, and a brownish dorsum with faint longitudinal black lines, whereas in *A. resplendens* the dorsum is dark gray with fine yellow stippling (Fig. 5b).

**Description of holotype.** Adult female, SVL 376 mm, tail length 37 mm (9.8% SVL); body diameter 9.1 mm; head length 11.7 mm (3.1% SVL); head width 6.4 mm (1.7% SVL); interocular distance 4.3 mm; head slightly distinct from body; snout-orbit distance 3.8 mm; rostral 2.5 mm wide, ca. as broad as high; internasals 1.3 mm wide; prefrontals 2.5 mm wide; frontal 3.1 mm wide, with a curvilinear triangular shape in dorsal view; parietals 2.4 mm wide (56% length); nasal divided; loreal 1.6 mm long, ~ 2 × longer than high; eye diameter 1.7 mm; pupil round; supraoculars 1.2 mm wide; two postoculars; temporals 1+2; seven supralabials, third and fourth contacting orbit; symphysial 1.7 mm wide, ~ 2 × as broad as long, separated from chinshields by first pair of infralabials; seven infralabials, first three contacting chin shields; chinshields ~ 2 × as long as broad, posterior chinshields absent; dorsal scales arranged in 17/17/17 rows, smooth without apical pits; two preventrals; ventrals 173; anal plate single; 25 paired subcaudals.

**Natural history.** Most individuals of *Atractus zgap* sp. nov. have been found during the day hidden under rocks, among herbs, or buried under soft soil in plantations and rural gardens close to remnants of native forest. At night, they have been seen crossing

**Figure 7.** Head of the adult female holotype of *Atractus zgap* sp. nov. ZSFQ 4946 in **a** dorsal **b** ventral **c** lateral right, and **d** lateral left view.

rural roads. Occasionally, during sunny days right after a rain, individuals have been seen crawling on the pavement or on gravel roads (Diego Piñán, pers. comm.).

**Distribution.** *Atractus zgap* sp. nov. is known only from five localities (See Suppl. material 1: Table S1) along the valley of the Río Quijos, Napo province, in the Amazonian slopes of the Andes in northeastern Ecuador, at elevations 1460–1703 m a.s.l. (Fig. 2).

**Etymology.** The specific epithet *zgap* is used as a noun in apposition and honors the 'Zoological Society for the Conservation of Species and Populations' (ZGAP) (https://www.zgap.de), a program seeking to conserve unknown but highly endangered species and their natural habitats throughout the world. The ZGAP grant program supports the fieldwork of young scientists who are eager to implement and start conservation projects in their home countries. Specifically, ZGAP has supported the work on endangered Andean reptiles in Ecuador conducted by AA and JV.

**Conservation status.** We consider *Atractus zgap* sp. nov. to be Endangered following the IUCN criteria B2a, b (i, iii) (IUCN 2001), because the species' extent of occurrence is estimated to be less than 500 $km^2$ (Fig. 2) and its habitat is severely fragmented and declining in extent and quality due to deforestation. The valley of the Río Quijos formed the eastern frontier of the Incan Empire (1400–1532) and the cloud forest in the area suffered from intensive land-use even before European arrival (Loughlin et al. 2018). Today, this valley is one of the most important cattle farming areas along the

eastern slopes of the Andes and the majority of the forest along the Quijos river plains has been destroyed. Although *A. zgap* occurs in one protected area (Bosque Protector "La Cascada") and its presence is expected in adjacent Parque Nacional Cayambe-Coca and Parque Nacional Sumaco Napo-Galeras, it has so far not been recorded in major protected areas.

***Atractus michaelsabini* sp. nov.**
https://zoobank.org/E85C68A2-DAEF-4BC5-A6B3-6D1FEDEB9983
Figs 8, 9, 10f–h
Proposed standard English name: Michael Sabin's Ground Snake.
Proposed standard Spanish name: Culebra tierrera de Michael Sabin.

*Atractus roulei* Savage, 1960: 68 (part).
*Atractus lehmanni* Arteaga et al., 2017: 97.

**Holotype.** ZSFQ 4938 (Figs 8, 9, 10g), adult male collected by Jorge Luis Romero at Corraleja, Azuay province, Ecuador (S3.3874, W79.22785; 2660 m).

**Paratypes.** MZUTI 5289, adult female collected by Jorge Luis Romero at the type locality. AMARU 002 (Fig. 10f), adult female collected by Jorge Luis Romero at the type locality. ZSFQ 4939 (Fig. 10h), juvenile female collected by Jose Vieira and Amanda Quezada at El Panecillo, El Oro province, Ecuador (S3.46753, W79.48248; 2750 m). QCAZ 7887 and 7902, adult male and female collected by Silvia Aldás in December 2006 at Guanazán, El Oro province, Ecuador (S3.44667, W79.49051; 2663 m). QCAZ 9643 and 9652, adult females collected by Silvia Aldás in August 2009 at El Panecillo, El Oro province, Ecuador (S3.46753, W79.48248; 2775 m). DHMECN 7644–45, adult males collected by Mario Yánez-Muñoz, Luis Oyagata, Patricia Bejarano, and Marco Altamirano in March 2010 at Reserva Biológica Yunguilla, Azuay province, Ecuador (S3.22684, W79.27520; 1748 m). AMNH 18325, adult female collected in July 1920 at El Chiral, El Oro province, Ecuador (S3.63825, W79.59723; 1841 m). AMNH 22110–11, collected in August 1921 at La Chonta, El Oro province, Ecuador (S3.56585, W79.85144; 1025 m).

**Diagnosis.** *Atractus michaelsabini* sp. nov. is placed in the genus *Atractus*, as diagnosed by Savage (1960), based on phylogenetic evidence (Fig. 1). The species is diagnosed based on the following combination of characters: (1) 15/15/15 smooth dorsals; (2) one postocular; (3) loreal 3 × longer than high; (4) temporals 1+2; (5) five or six supralabials, with (usually) third and fourth contacting orbit; (6) five or six infralabials, with (usually) first three contacting chinshields; (7) 9–13 maxillary teeth; (8) 1–3 rows of gular scales; (9) 1–3 preventrals; (10) 143–144 ventrals in males and 144–153 in females; (11) 24–31 subcaudals in males and 17–19 in females; (12) dorsal ground color golden yellow (Figs 8, 10f–g) to dark brown (Fig. 10h) with each scale outlined in black, forming a reticulation; (13) venter yellowish with various degrees of brown stippling (Fig. 8b); (14) 256–321 mm

**Figure 8.** Adult male holotype of *Atractus michaelsabini* sp. nov. ZSFQ 4938 in **a** dorsal and **b** ventral view.

SVL in males and 201–392 mm SVL in females; (15) 35–42 mm TL in males and 21–37 mm TL in females.

**Comparisons.** *Atractus michaelsabini* sp. nov. is compared to other members of the *A. roulei* species group: *Atractus carrioni* and *A. roulei*. From *A. carrioni*, the new species differs in having a loreal scale (Fig. 9c) (absent in *A. carrioni*). From *A. roulei* (Figs 10a–e), the new species differs in having a dorsal pattern in which each scale is outlined in a thin black line, thus creating a reticulation, and by having the prefrontal scale in broad contact with the postnasal (Fig. 9c) (not in contact or barely in contact in *A. roulei*). Furthermore, the existence of the bright golden yellow morph in adult individuals has so far been recorded only in *A. michaelsabini* sp. nov.; not in *A. roulei*, where adults are dark brown dorsally (Fig. 10a–e). In *A. roulei*, there is a black spot at the base of each dorsal scale, whereas in *A. michaelsabini* sp. nov. the spot is at the tip of each dorsal scale and is connected to the black reticulum. Genetic divergence in a 578 bp long fragment of the mitochondrial CYTB gene between *A. michaelsabini* sp. nov. and *A. roulei* is 6.5–7.2%, whereas intraspecific distances are 0–4.5% in *A. michaelsabini* sp. nov. and 0–4.8% in *A. roulei*.

**Description of holotype.** Adult male, SVL 256 mm, tail length 39 mm (15.2% SVL); body diameter 7.4 mm; head length 10.7 mm (3.1% SVL); head width 6.4 mm (2.5% SVL); interocular distance 3.7 mm; head slightly distinct from body; snout-orbit distance 3.5 mm; rostral 1.9 mm wide, ca. as broad as high; internasals 1.0 mm wide; prefrontals 2.0 mm wide; frontal 3.0 mm wide, with a curvilinear triangular shape in dorsal view; parietals 2.9 mm wide (65% length); nasal divided; loreal

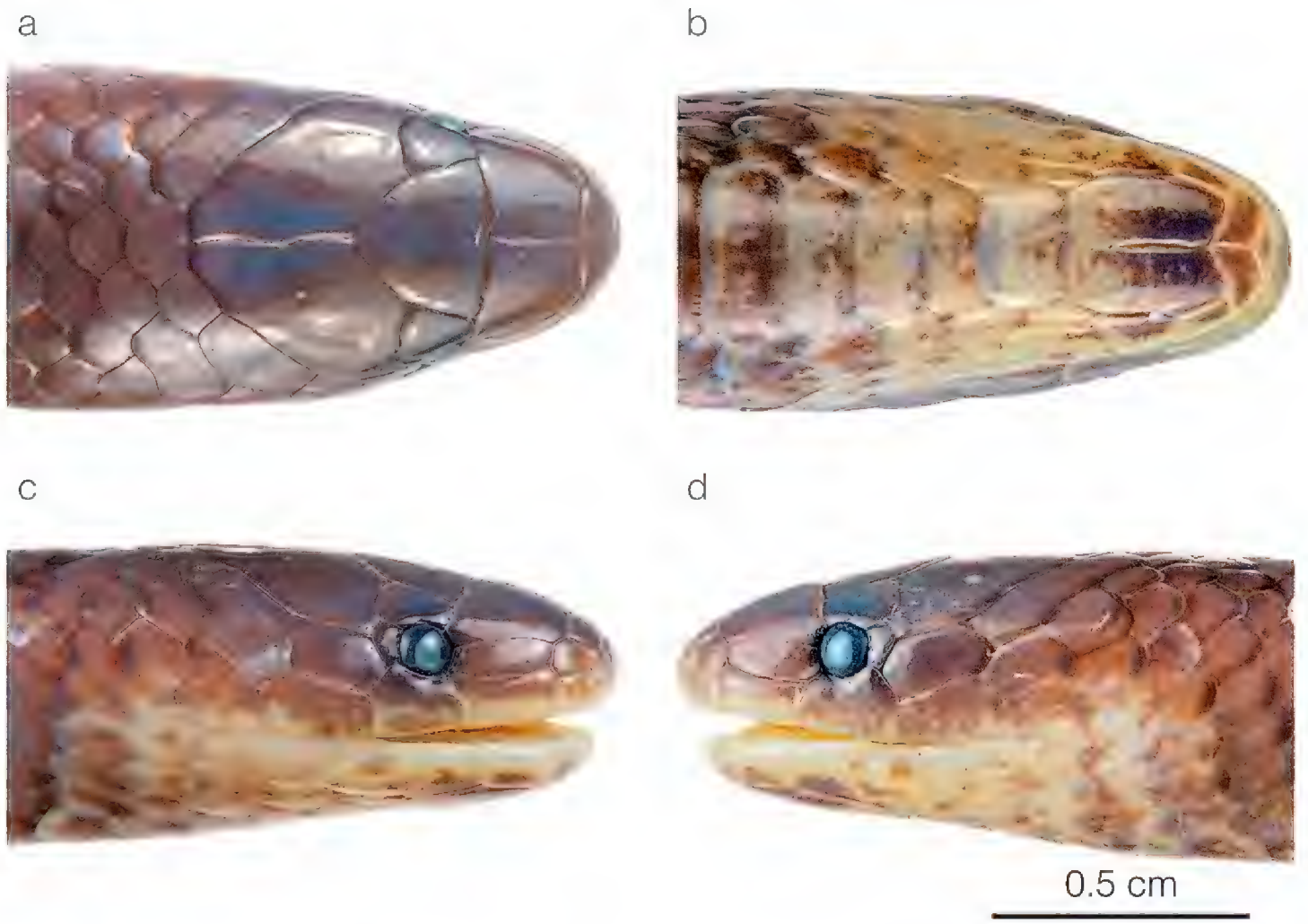


**Figure 9.** Head of the adult male holotype of *Atractus michaelsabini* sp. nov. ZSFQ 4938 in **a** dorsal **b** ventral **c** lateral right, and **d** lateral left view.

2.2 mm long, ~ 3 × longer than high; eye diameter 1.4 mm; pupil round; supraoculars 1.3 mm wide; one postocular; temporals 1+2; five supralabials, third contacting orbit; symphysial 1.7 mm wide, ~ 3 × as broad as long, separated from chinshields by first pair of infralabials; five infralabials, first three contacting chinshields; chinshields ~ 2 × as long as broad, posterior chinshields absent; dorsal scales arranged in 15/15/15 rows, smooth without apical pits; no preventrals; ventrals 143; anal plate single; 31 paired subcaudals.

**Natural history.** Most individuals of *Atractus michaelsabini* sp. nov. have been found during the day hidden under rocks, mats of rotten vegetation, or buried in soft soil in pastures and maize plantations close to remnants of native forest. At night, they have been seen crossing forest trails. At the type locality, clutches of three or four eggs have been found under soil (Jorge Luis Romero, pers. comm.). Anecdotal information suggests that these snakes are more active during the rainy months (February-May at the type locality; Jorge Luis Romero, pers. comm.).

**Distribution.** *Atractus michaelsabini* sp. nov. is endemic to an estimated 2,530 $km^2$ area along the Pacific slopes of the Andes in southwestern Ecuador. The species occurs in the xeric inter-Andean valley of the Río Jubones as well as on the slopes of the Cordillera de Chilla. *Atractus michaelsabini* sp. nov. is known from provinces Azuay, El Oro, and Loja, and has been recorded at elevations between 927 and 2922 a.s.l. (Fig. 2).

**Figure 10.** Photographs of living specimens of *Atractus roulei* and *A. michaelsabini* sp. nov. **a** *A. roulei* ZSFQ 4942 from Tixán, Chimborazo province, Ecuador **b** *A. roulei* ZSFQ 4944 from Tixán, Chimborazo province, Ecuador **c** *A. roulei* ZSFQ 4941 from Tixán, Chimborazo province, Ecuador **d** *A. roulei* ZSFQ 4945 from Tixán, Chimborazo province, Ecuador **e** *A. roulei* from Tixán, Chimborazo province, Ecuador **f** *A. michaelsabini* sp. nov. AMARU 002 from Corraleja, Azuay province, Ecuador **g** *A. michaelsabini* sp. nov. holotype ZSFQ 4938 from Corraleja, Azuay province, Ecuador and **h** *A. michaelsabini* sp. nov. ZSFQ 4939 from El Panecillo, El Oro province, Ecuador.

**Etymology.** The specific epithet *michaelsabini* is a patronym honoring a young nature lover, Michael Sabin, grandson of American philanthropist and conservationist Andrew "Andy" Sabin. The Sabin family is involved in conservation and field research of amphibians and reptiles and has protected over 264,365 acres of critical habitat throughout the world.

**Conservation status.** We consider *Atractus michaelsabini* sp. nov. to be Endangered following the IUCN criteria B1a, b (i, iii) (IUCN 2001), because the species' extent of occurrence is estimated to be much less than 5,000 km$^2$ (Fig. 2) and its habitat is severely fragmented and declining in extent and quality due to deforestation. Although *A. michaelsabini* sp. nov. is present in two protected areas (private reserves Buenaventura and Yunguilla of Fundación Jocotoco), nine of the 14 localities where the species has been recorded (Suppl. material 1: Table S1) are in heavily human-modified areas. Based on maps of Ecuador's vegetation cover (MAE 2012), we estimate that nearly 70% of the forest cover throughout the species' potential distribution area has been destroyed, mostly due to the expansion of the agricultural frontier.

## Distribution maps

Our resulting distribution maps increase the number of known localities of occurrence for the studied taxa (listed under Suppl. material 1: Table S1) and show a distinct geographical separation between *Atractus roulei* and *A. michaelsabini* sp. nov. (Fig. 2). The predicted area of suitable habitat for *A. michaelsabini* sp. nov. includes the upper watershed of the Río Jubones (a xeric inter-Andean valley) as well as both slopes of the Cordillera de Chilla (an area having vegetation classified as evergreen montane forest; see Sierra 1999). Likewise, the predicted area of suitable habitat for *A. roulei* includes evergreen montane forests along the Pacific slopes of the Andes as well as the xeric inter-Andean valley of the upper Río Chanchán. The predicted area of suitable habitat for *A. arangoi* includes almost the entire extent of Pastaza province, although we did not find records of this species from this province. Although we did not build binary environmental niche models for *A. discovery* sp. nov. and *A. zgap* sp. nov. (only two and six localities are available for these species), they are both known only from their corresponding river valleys and occur on both sides of the Río Paute and Río Quijos, respectively.

## Revalidation of *Atractus arangoi*

Prado (1939) described *Atractus arangoi* from Colombia whereas Daniel (1949) reported this species in Puerto Asís, Putumayo department. Schargel et al. (2013) considered *A. arangoi* to be a junior synonym of *A. major* claiming that all the putative diagnostic characters for *A. arangoi* fall within the variation in *A. major* as defined in their work. In our phylogenetic tree of *Atractus* (Fig. 1), we included sequences of three snakes that fit the original description of *A. arangoi*. DHMECN 8343 (reported as *A. major* in Arteaga et al. 2017), ZSFQ 4947 (Fig. 11), and ZSFQ 4948 (Fig. 5a). These three specimens form a strongly supported clade sister to all other samples of *A. major*, which includes specimens from throughout the latter species' area of distribution. Furthermore, we find

that these specimens, in addition to others reported in the literature as *A. torquatus* and *A. major* (see Duellman 1978; Maynard et al. 2017) can easily be separated from *A. major* based on differences in coloration, body size (compare Figs 5a and 5i, j), and ventral and subcaudal counts (summarized in Table 3), as originally suggested by Prado (1939). Thus, we formally remove *A. arangoi* from the synonymy of *A. major*, include this species in the herpetofauna of Ecuador, and provide a distribution map for this species (Fig. 2).

## Presence of *Atractus gigas* in Peru

Passos et al. (2010) reported *Atractus gigas*, a snake species previously considered to be endemic to the cloud forests of northwestern Ecuador (Myers and Schargel 2006), on the Amazonian slopes of the Andes in Peru. The identification of the Peruvian specimens as *A. gigas* was based on their large size and the partial overlap in some characters of lepidosis with the Ecuadorian samples. However, these Peruvian snakes have a smaller number of subcaudals (25 or 26 instead of 31–37 in Ecuadorian specimens), a shorter loreal scale, first four infralabials contacting chinshields (instead of first three in Ecuadorian specimens), and a completely different color pattern in both juveniles and adults (for a figure depicting the variation among Ecuadorian individuals see Arteaga 2022). Juveniles of "*A. gigas*" from Peru have a black dorsum with short (one scale wide) reddish brown bands whereas juveniles of Ecuadorian *A. gigas* have a contrasting pattern of dark-brown to black rounded bands or blotches on a rosy white background color. Adults of "*A. gigas*" from Peru have a dorsal pattern in which each scale is dark brown distally but cream towards the base, forming a reticulation. Adults of *A. gigas* from Ecuador are uniformly rich dark brown or glossy black, and the skin between the scales is whitish (Arteaga 2022). QCAZ 14946, a specimen identified as *A. atlas* in Melo-Sampaio et al. (2021) from Reserva Biológica Cerro Plateado, just 7 km from the Peruvian border on the southeastern slopes of the Ecuadorian Andes, resembles Peruvian "*A. gigas*" as depicted in Passos et al. (2010) in having a short loreal, dorsal scales with a cream base, first four infralabials contacting chinshields, and fewer than 30 subcaudals. This specimen was included in our phylogeny (Fig. 1) and was recovered as the strongly supported sister taxon to a new sample of *A. touzeti* from this species' type locality. Based on this evidence, we suggest that Peruvian specimens CORBIDI 877 and ZFMK 89147, as well as other *Atractus* specimens from Cajamarca labeled as *A. gigas*,

**Table 3.** Differences in coloration, scale counts, and size between *Atractus arangoi* and *A. major*. The range of each continuous variable is from our own sample, Prado (1939), and Maynard et al. (2017). The numbers in parentheses represent the sample size.

| Variable character | *Atractus arangoi* | | *Atractus major* | |
|---|---|---|---|---|
| Dark brown or black nape stripe | Absent | | Present | |
| Dorsal markings | Irregular dark blotches | | Complete irregular dark bands anteriorly; blotches posteriorly | |
| Sex | Males ($n$ = 2) | Females ($n$ = 2) | Males ($n$ = 7) | Females ($n$ = 5) |
| Maximum SVL | 309 mm | 412 mm | 533 mm | 986 mm |
| Ventral scales | 154–163 | 160–161 | 162–165 | 172–177 |
| Subcaudal scales | 38–39 | 29–32 | 36–45 | 34–37 |

**Figure 11.** Adult male of *Atractus arangoi* ZSFQ 4947 in **a** dorsal and **b** ventral view.

be reidentified as *A. atlas*, or at the very least, be considered as an undescribed species related to the latter. Thus, we suggest *A. gigas* be removed from the herpetofauna of Peru, a view that confirms this species as endemic to the cloud forests of northwestern Ecuador as originally suggested by Myers and Schargel (2006) and Arteaga et al. (2013).

## Status of *Atractus occidentalis* and reidentification of specimens of *Atractus* of the iridescens group

In his unpublished BSc thesis, Mejía Guerrero (2018) used species distribution models, a comprehensive (based on 88 specimens) comparison of scale counts, and species delimitation analysis based on a combination of novel DNA sequences and those provided in Arteaga et al. (2017) to test species limits within the *Atractus iridescens* species group. He proposed that *A. occidentalis* Savage, 1955 is a junior synonym of *A. microrhynchus* and that some individuals identified as *A. dunni* from Mindo are actually *A. microrhynchus*. The topology for the included members of the *A. iridescens* group in our BI phylogeny (Fig. 1) and that of Murphy et al. (2019), though not identical, agree with the proposal of Mejía Guerrero (2018). Based this evidence, we also consider *A. occidentalis* to be a junior synonym of *A. microrhynchus*. Recently, Passos et al. (2022) provided a list of reidentifications of 15 (not 17, because two are duplicates and MZUTI 4178 retained the same identification despite being listed in the table) *Atractus* specimens having sequences deposited in GenBank, notably among them the members of the *A. iridescens* species group deposited in MZUTI and DHMECN. In this work, one reidentification (that of the holotype of *A. pyroni*; MZUTI 5107) was backed up by ample evidence and two oth-

ers (ANF 2390, now MZUTI 5409; and GFM 307, now MPEG 21582) were substantiated in Melo-Sampaio et al. (2021), but the remaining were proposed without providing any evidence, either in the form of new phylogenetic relationships, new scale counts, or previously unsampled morphological features. Since these specimens are deposited at MZUTI and DHMECN, as well as their corresponding photo vouchers available in Arteaga et al. (2017), and their DNA sequences on GenBank, their identity can be tested by anyone. Although the reidentification of the remaining specimens provided by Passos et al. (2022) was unsubstantiated, not all of them were unwarranted (see Table 4). We agree that DHMECN 7644 (identified as *A. lehmanni* Boettger, 1898 in Arteaga et al. 2017) and IBSP 71932 (identified as *A. zebrinus* Jan, 1862 in Grazziotin et al. 2012) are misidentified, but their new identifications provided by Passos et al. (2022) are not correct either (see Table 4). DHMECN 7644 is a paratype of *A. michaelsabini* sp. nov., as defined herein, and IBSP 71932 is probably an *A. trihedrurus* Amaral, 1926, not an "*A. triherurus*." Although the latter probably represents a typo and is a minor error, the problems with the remaining reidentifications are not trivial. For example, Passos et al. (2022) reidentified the same specimen, MZUTI 3758, as *A. iridescens* Peracca, 1896 and also as *A.* cf. *iridescens*. Additionally, these authors completely reidentified the type series of both *A. cerberus* and *A. esepe* Arteaga et al., 2017, probably without much confidence since this action is not explained elsewhere in their work and is not trivial. Since MZUTI 4330 and MZUTI 3758 are name-bearing specimens, reidentification of these holotypes as *A. iridescens*, *A.* cf. *iridescens*, or anything other than their original identification presented in Arteaga et al. (2017) implies that these species are not valid. Surprisingly, the fact that the taxonomic validity of these two species is not questioned elsewhere in Passos et al. (2022) suggests that some of these reidentifications were proposed carelessly. Thus, in Table 4, we evaluate these reidentifications and mention whether they are substantiated or warranted or neither. Finally, we propose the reidentification of an additional six *Atractus* specimens (Table 5) having sequences deposited in GenBank based on the results presented in Fig 1.

**Table 4.** Reidentification of *Atractus* specimens reidentified in Passos et al. 2022 based on direct examination of voucher specimens.

| Voucher | Original identification (Arteaga et al. 2017) | Proposed reidentification (Passos et al 2022) | Reidentification warranted and substantiated | Identification |
|---|---|---|---|---|
| MZUTI 4330 | *Atractus cerberus* | *Atractus* cf. *iridescens* | No | *Atractus cerberus* |
| MZUTI 1385, 2649–50, 3323 | *Atractus occidentalis* | *Atractus dunni* | No | *Atractus microrhynchus* |
| MZUTI 3758–59 | *Atractus esepe* | *Atractus* cf. *iridescens* and *A. iridescens* | No | *Atractus esepe* |
| MZUTI 4178 | *Atractus iridescens* | *Atractus iridescens* | Identity remained the same, but listed as "reidentified" | *Atractus iridescens* |
| MZUTI 4122 | *Atractus microrhynchus* | *Atractus iridescens* | No | *Atractus microrhynchus* |
| DHMECN 7644 | *Atractus lehmanni* | *Atractus roulei* | Warranted at time of publication | *Atractus michaelsabini* sp. nov. |
| MZUTI 5109 | *Atractus microrhynchus* | *Atractus dunni* | No | *Atractus microrhynchus* |
| MZUTI 5107 | *Atractus pyroni* | *Atractus roulei* | Yes | *Atractus roulei* |
| ANF 2390 | *Atractus touzeti* | *Atractus pachacamac* | Yes | *Atractus pachacamac* |
| GFM 307 | *Atractus schach* | *Atractus snethlageae* | Yes | *Atractus snethlageae* |
| IBSP 71932 | *Atractus zebrinus* | *Atractus triherurus* | Yes, but name misspelled | *Atractus trihedrurus* |

**Table 5.** Reidentification of *Atractus* sequences available in GenBank based on direct examination of voucher specimens.

| Voucher | GenBank accession numbers | Identity in GenBank | Identification |
|---|---|---|---|
| DHMECN 8343 | KY610059, KY610105 | *Atractus major* | *Atractus arangoi* |
| QCAZ 7887 | MT507872, MT511989 | *Atractus roulei* | *Atractus michaelsabini* sp. nov. |
| QCAZ 7889 | MT507874, MT511990 | *Atractus roulei* | *Atractus michaelsabini* sp. nov. |
| QCAZ 9643 | MT507875, MT511981, MT511991 | *Atractus roulei* | *Atractus michaelsabini* sp. nov. |
| QCAZ 9652 | MT507876, MT511992 | *Atractus roulei* | *Atractus michaelsabini* sp. nov. |
| MHUA 14368 | GQ334664, GQ334581, GQ334558, GQ334480 | *Atractus wagleri* | *Atractus lasallei* |

## Discussion

*Atractus* is perhaps the most taxonomically complex snake genus and the work needed to elucidate its evolutionary relationships is just starting. Achieving a comprehensive understanding of the real diversity within this cryptozoic group of snakes will require an approach combining three actions: 1) improving the taxon sampling available for comparison at the molecular level; 2) re-sampling type localities as well as exploring new remote areas; and 3) defining species boundaries among *Atractus* species using an integrative taxonomic approach, not only scale counts. Below, we discuss how our results help clear the waters in *Atractus* taxonomy and provide insights on where future research efforts might be most effective.

The molecular phylogenies presented here (Fig. 1 and Suppl. material 2: Fig. S1) include only approximately 30% of the total known diversity of the genus *Atractus*; thus, many higher-level relationships within species groups are still unknown. The placement of *A. trilineatus* as sister to a clade containing *A. arangoi* and *A. major*, rather than as an early divergent *Atractus* species (Murphy et al. 2019) is puzzling, but this relationship is moderately supported in both the BI and ML analyses and will likely benefit from an improved sampling of molecular characters. *Atractus arangoi* is supported as a valid species in our molecular analyses and is easily diagnosable from *A. major* based on body size, coloration, and lepidosis (Table 3), confirming its status as a valid species (Prado 1939; Daniel 1949). With the exception of the weakly placed *A. zidoki* Gasc & Rodrigues, 1979, we found that cis-Andean species of *Atractus* are more closely related to other cis-Andean species, whereas trans-Andean ground snakes are more closely related to other trans-Andean species. This finding may prove useful in understanding why the presence of the same *Atractus* species on both sides of the Andes, a scenario suggested for *A. gigas* by Passos et al. (2010), is unlikely.

There is a clade formed by the remaining Ecuadorian *Atractus* that were included in the phylogeny and are distributed along the Amazonian slopes of the Andes. The new species, *A. discovery* sp. nov. and *A. zgap* sp. nov., are included in this group. While the former is the strongly supported sister species to *A. resplendens*, it has a coloration pattern most similar to *A. orcesi* (Fig. 5e), a species not previously included in any phylogenetic analyses and characterized by having a yellow belly with a black ventral stripe. The black stripe on a yellow belly is a characteristic shared by *A. duboisi*, *A. discovery* sp. nov., and *A. orcesi*, but is absent from *A. resplendens* and *A. ecuadorensis* (the other two members of the group) and confirms this as a useful character in diagnosing species within this clade. In the ML analysis (Suppl. material 2: Figure S1), *A. dunni* is nested within

*A. microrhynchus*, a topology not recovered in the BI phylogeny or in previous analyses despite being based on the same DNA sequences. We believe this incongruence is the result of character sampling and methodological approach instead of these two species being conspecific. The phylogenetic position of *A. zgap* sp. nov., a snake most similar to *A. ecuadorensis* in size, coloration, and lepidosis, as sister to a clade of banded Amazonian *Atractus* rather than to *A. ecuadorensis* is puzzling. Although the placement of *A. zgap* sp. nov. in both the BI and ML analyses is strongly supported and is probably correct, we do not have as much confidence in the position of *A. ecuadorensis* and this may be explained by the fact that only one gene fragment (ND4) was available for the latter species (Appendix I). We found higher intraspecific topological distances between members of *A. carrioni*, *A. major*, and *A. roulei* than between the pair of species *A. trefauti-A. schach*. Therefore, attention should be given to reevaluating the validity of these species.

The binary environmental niche models (Fig. 2) for both *Atractus michaelsabini* sp. nov. and *A. roulei* include xeric inter-Andean valleys where populations of these snakes are known to occur, even though elsewhere these species inhabit humid areas where the dominant vegetation cover is evergreen montane forest (Sierra 1999). We found that the deep intraspecific genetic divergence found within both of these taxa corresponds to the sampling of populations distributed on different bioclimatic regimes (i.e., snakes of xeric habitats are genetically distinct from snakes of humid habitats). Although we did not find morphological differences that would allow the distinction of these subpopulations, we do not rule out the possibility that they correspond to cryptic species diversity.

In addition to creating a more robust phylogenetic tree of ground snakes, one of the most important actions in the quest towards a more clear, stable, and useful *Atractus* taxonomy is the correct identification of museum specimens. Based on our review of the reidentifications proposed in Passos et al. (2022), it is evident that reassigning the species identities of museum vouchers is not a trivial pursuit. On the contrary, it has consequences that go beyond taxonomy. For example, reidentifying the only known museum specimens of the Critically Endangered *A. cerberus* as *A. iridescens*, a Least Concern species, implies that the population of this species in the isolated Pacoche forest of west-central Ecuador is not as unique and worthy of conservation efforts. It also implies that the presence of a species endemic to the humid Chocó rainforest in an isolated mountain range belonging to another biogeographic province is likely.

The last point on biogeography deserves elaboration. The use of species distribution models can be used not only to discover and test biogeographical patters but also to test species as hypotheses (Ahmadzadeh et al. 2013; Ortega-Andrade et al. 2015). The elaboration of distribution maps using ecological variables, in addition to the presentation of accurate color photographs of specimens and their corresponding genetic information as a part of an integrative taxonomic approach can greatly benefit *Atractus* taxonomy, a branch of herpetology in which diagnoses have largely been based only on meristics (Savage 1960; Passos et al. 2009c; Passos et al. 2010). Using this framework can help prevent *Atractus* species that are valid taxa and occur in distinct biogeographical provinces to be subsumed under the same name on the basis of overlapping scale counts. An example of this are the snakes *A. gigas* and *A. dunni*, two cloud forest species endemic to the Pacific slopes of the Andes in northwestern Ecuador. These snakes

present a biogeographic pattern of distribution shared by other co-occurring reptiles (Avila Pires 2001; Köhler et al. 2004; Arteaga et al. 2013; Torres-Carvajal and Lobos 2014; Arteaga et al. 2016). Given how narrow the climatic requirements of these two *Atractus* species are (Mejía Guerrero 2018; Mantilla Espinoza 2021), their presence on the Amazonian slopes of the Andes, or on the Chocoan lowlands, as suggested by Passos et al. (2010) and Passos et al. (2022), respectively, is unlikely. In this work, we presented evidence that supports the status of *A. gigas* and *A. dunni* as species endemic to the cloud forests of the Pacific slopes of the Andes in northwestern Ecuador.

Finally, although *Atractus* systematics have progressed greatly since Savage published his monograph on the Ecuadorian members of this genus in 1960, many "stones are still left unturned." The Ecuadorian species *A. clarki* Dunn & Bailey, 1939, *A. collaris* Peracca, 1897, *A. gaigeae* Savage, 1955, and *A. occipitoalbus* have not been included in a phylogenetic work, and their status remains uncertain. Also, an overwhelming majority of *Atractus* diversity, both described and undescribed, is in Colombia (Uetz et al. 2022). Unfortunately, only one or two samples of *Atractus* coming from Colombia have been included in published phylogenetic trees of this genus (Arteaga et al. 2017; Murphy et al. 2019; Melo-Sampaio et al. 2021, Passos et al. 2022). Thus, we suggest that future work on *Atractus* be focused on unveiling the incredible diversity of this genus in Colombia.

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## Appendix I

**Table A1.** GenBank accession numbers for loci and terminals of taxa and outgroups sampled in this study. Novel sequence data produced in this study are marked with an asterisk (*).

| Species | Voucher | 16S | CYTB | ND4 | CMOS | NT3 | RAG1 |
|---|---|---|---|---|---|---|---|
| *A. arangoi* | DHMECN 8343 | KY610059 | – | KY610105 | – | – | – |
| *A. arangoi* | ZSFQ 4947 | ON907812* | ON925021* | ON925012* | – | – | – |
| *A. arangoi* | ZSFQ 4948 | ON907811* | ON925020* | ON925011* | – | – | – |
| *A. atlas* | QCAZ 14946 | MH790470 | MN887669 | MN887691 | MN887640 | MN887715 | MN887745 |
| *A. badius* | MNRJ 26717 | MH790476 | MK835891 | – | MK835864 | MK835980 | MK835948 |
| *A. boimirim* | MPEG 21233 | MH790478 | – | – | MK835866 | MK835982 | MK835951 |
| *A. carrioni* | MZUTI 4195 | KY610046 | – | KY610094 | – | – | – |
| *A. carrioni* | QCAZ 6446 | MT507867 | – | MT511983 | – | – | – |
| *A. carrioni* | QCAZ 6533 | MT507868 | – | MT511984 | – | – | – |
| *A. carrioni* | QCAZ 6534 | MT507869 | – | MT511985 | – | – | – |
| *A. carrioni* | QCAZ 10038 | MT507864 | MT511977 | MT511982 | – | – | – |
| *A. carrioni* | QCAZ 13094 | MT507865 | MT511978 | – | – | – | – |
| *A. cerberus* | MZUTI 4330 | KY610047 | KY610073 | KY610095 | – | – | – |
| *A. dapsilis* | MNRJ 16796 | MH790480 | MK835894 | MK835926 | MN887642 | MN887716 | MK835951 |
| *A. discovery* sp. nov. | MZUA.Re.466 | OP225330* | OP244686* | OP225393* | – | – | – |
| *A. duboisi* | MZUTI 62 | KT944041 | – | KT944059 | – | – | – |
| *A. dunni* | MZUTI 2189 | KY610048 | – | KY610096 | – | – | – |
| *A. dunni* | MZUTI 3031 | KY610049 | – | KY610097 | – | – | – |
| *A. dunni* | MZUTI 4318 | KY610050 | KY610074 | KY610098 | – | – | – |
| *A. dunni* | MZUTI 4319 | KY610051 | KY610075 | KY610099 | – | – | – |
| *A. ecuadorensis* | DHMECN 5105 | – | – | KY610100 | – | – | – |
| *A. elaps* | QCAZ 5574 | MN855378 | MK835896 | MN887692 | MK835867 | MN887717 | MK835954 |
| *A. esepe* | MZUTI 3758 | KY610053 | KT944052 | KY610102 | – | – | – |
| *A. esepe* | MZUTI 3759 | KT944039 | KT944051 | KT944058 | – | – | – |
| *A. favae* | MZUSP 20211 | MN855380 | MN887670 | – | – | – | – |
| *A. flammigerus* | MNRJ 26720 | MH790488 | MK835903 | MK835932 | MK835873 | MK835994 | – |
| *A. gigas* | MZUTI 3286 | KT944043 | KT944053 | MN891764 | – | – | – |
| *A. iridescens* | DHMECN 9633 | KY610054 | KY610077 | – | – | – | – |

| Species | Voucher | 16S | CYTB | ND4 | CMOS | NT3 | RAG1 |
|---|---|---|---|---|---|---|---|
| *A. iridescens* | MZUTI 3548 | KY610055 | KY610078 | – | – | – | – |
| *A. iridescens* | MZUTI 3680 | KY610056 | KY610079 | – | – | – | – |
| *A. iridescens* | MZUTI 4178 | KT944040 | KY610080 | – | MH374931 | – | – |
| *A. iridescens* | MZUTI 4697 | KY610057 | KY610081 | – | – | – | – |
| *A. lasallei* | MHUA 14368 | – | GQ334480 | GQ334581 | – | – | – |
| *A. latifrons* | MPEG 22630 | MH790493 | MK835908 | MN887694 | MK835875 | – | – |
| *A. major* | ANF 1545 | KT944045 | – | KY610104 | – | – | – |
| *A. major* | CORBIDI 223 | MH790497 | – | – | – | – | – |
| *A. major* | MNRJ 26126 | MH790498 | MK835911 | – | – | – | MK835958 |
| *A. major* | MZUSP 20868 | MH790499 | – | – | – | – | – |
| *A. major* | MZUSP 20887 | MH790500 | – | – | – | – | – |
| *A. major* | QCAZ 4691 | MH790506 | MK835912 | MK835934 | MN887643 | MK836002 | MN887747 |
| *A. major* | QCAZ 4993 | MH790507 | – | MK835935 | – | – | – |
| *A. major* | QCAZ 5891 | MH790508 | MK835913 | MK835936 | MK835878 | MK836003 | MK835962 |
| *A. major* | QCAZ 7881 | MH790509 | MK835914 | MK835937 | – | MK836004 | MK835963 |
| *A. major* | QCAZ 13819 | MH790504 | – | MK835933 | – | MK836000 | MK835960 |
| *A. major* | UFACRB 532 | MH790511 | MK835915 | – | MK835879 | MK836005 | – |
| *A. michaelsabini* sp. nov. | AMARU 002 | ON907809* | ON925018* | ON925009* | – | – | – |
| *A. michaelsabini* sp. nov. | MZUTI 5289 | ON907810* | ON925019* | ON925010* | – | – | – |
| *A. michaelsabini* sp. nov. | DHMECN 7644 | KY610058 | KY610082 | KY610103 | – | – | – |
| *A. michaelsabini* sp. nov. | QCAZ 7887 | MT507872 | – | MT511989 | – | – | – |
| *A. michaelsabini* sp. nov. | QCAZ 7889 | MT507874 | – | MT511990 | – | – | – |
| *A. michaelsabini* sp. nov. | QCAZ 9643 | MT507875 | MT511981 | MT511991 | – | – | – |
| *A. michaelsabini* sp. nov. | QCAZ 9652 | MT507876 | – | MT511992 | – | – | – |
| *A. michaelsabini* sp. nov. | ZSFQ 4939 | ON907808* | ON925017* | ON925008* | – | – | – |
| *A. microrhynchus* | MZUTI 1385 | KY610063 | KY610086 | KY610109 | – | – | – |
| *A. microrhynchus* | MZUTI 2649 | KY610064 | KY610087 | KY610110 | – | – | – |
| *A. microrhynchus* | MZUTI 2650 | KT944038 | KT944050 | KT944057 | – | – | – |
| *A. microrhynchus* | MZUTI 3323 | KY610065 | KY610088 | KY610111 | – | – | – |
| *A. microrhynchus* | MZUTI 4122 | KT944037 | KT944049 | KT944056 | – | – | – |
| *A. microrhynchus* | MZUTI 5109 | KY610060 | KY610083 | KY610106 | – | – | – |
| *A. modestus* | MZUTI 4760 | KY610061 | KY610084 | KY610107 | – | – | – |
| *A. multicinctus* | MZUTI 5106 | KY610062 | KY610085 | KY610108 | – | – | – |
| *A. orcesi* | ZSFQ 2222 | ON907807* | – | ON925007* | – | – | – |
| *A. orcesi* | ZSFQ 2237 | ON907806* | ON925016* | ON925006* | – | – | – |
| *A. pachacamac* | QCAZ 12630 | MH790524 | MN887672 | MN887697 | MN887647 | MN887723 | MN887751 |
| *A. paucidens* | MZUTI 5102 | KY610066 | ON925015* | KY610112 | – | – | – |
| *A. resplendens* | MZUTI 3996 | KT944042 | KT944055 | KT944060 | – | – | – |
| *A. riveroi* | MNRJ 26087 | MH790526 | MK835916 | – | – | MK836006 | MK835964 |
| *A. roulei* | MZUTI 4503 | KY610069 | KY610090 | KY610116 | – | – | – |
| *A. roulei* | MZUTI 4544 | KY610069 | KY610091 | KY610117 | – | – | – |
| *A. roulei* | MZUTI 5107 | KY610068 | KY610089 | KY610115 | – | – | – |
| *A. roulei* | QCAZ 6256 | – | MT511980 | MT511988 | – | – | – |
| *A. roulei* | QCAZ 7192 | MT507871 | MT511980 | – | – | – | – |
| *A. roulei* | ZSFQ 4945 | ON907805* | ON925014* | ON925005* | – | – | – |
| *A. savagei* | MZUTI 4916 | KY610070 | KY610092 | KY610118 | – | – | – |
| *A. schach* | AF 1716 | MH790527 | MK835917 | – | MK835880 | MK836007 | – |
| *A. snethlageae* | MPEG 20605 | MH790513 | MN887678 | MN887705 | MN887655 | MN887731 | MN887759 |
| *A. tartarus* | MPEG 23931 | MH790529 | MK835919 | MK835938 | – | MK836009 | MK835965 |
| *A. torquatus* | MPEG 23686 | MH790532 | MK835921 | MK835941 | – | MK836012 | MK835968 |
| *A. touzeti* | ZSFQ 4949 | ON907804* | ON925013* | ON925004* | – | – | – |
| *A. trefauti* | MNRJ 26709 | MH790536 | MK835923 | MK835942 | MK835883 | MK836015 | MK835971 |
| *A. trilineatus* | CAS 257740 | MK648018 | MK648027 | MK648035 | MK648043 | – | – |
| *A. trilineatus* | UWISM 2015.18.2 | MK648014 | MK648022 | MK648031 | MK648039 | – | – |
| *A. typhon* | MZUTI 3284 | KT944044 | KT944054 | KT944062 | – | – | – |

| Species | Voucher | 16S | CYTB | ND4 | CMOS | NT3 | RAG1 |
|---|---|---|---|---|---|---|---|
| *A. ukupacha* | QCAZ 4944 | MH790540 | MN887689 | MN887714 | MN887668 | MN887744 | MN887774 |
| *A. zgap* sp. nov. | MZUTI 5311 | ON907803* | – | ON925003* | – | – | – |
| *A. zidoki* | MNHN 1997.2046 | AF158487 | – | – | – | – | – |
| *G. godmani* | MVZ 233298 | JQ598877 | JQ598932 | – | – | – | – |
| *S. nebulatus* | MVZ 233298 | EU728583 | EU728583 | EU728583 | – | – | – |

# Appendix II

**Table A2.** List of PCR and sequencing primers and their respective PCR conditions (denaturation, annealing, extension, and number of corresponding cycles) used in this study. All PCR protocols included an initial 3-min step at 94 °C and a final extension of 10 min at 72 °C.

| Locus | Primer | Sequence (5'-3') | Reference | PCR profile |
|---|---|---|---|---|
| 16S | 16Sar-L | CGCCTGTTTATCAAAAACAT | Palumbi et al. (1991) | 30 cycles of 94 °C (45 sec), 53 °C (45 sec), 72 °C (1 min) |
| | 16Sbr-H-R | CCGGTCTGAACTCAGATCACGT | | |
| Cytb | L14910 | GACCTGTGATMTGAAAACCAYCGTTGT | Burbrink et al. (2000) | 94 °C (1 min), 58 °C (1 min), 72 °C (2 min) [x30–36] |
| | H16064 | CTTTGGTTTACAAGAACAATGCTTTA | | |
| ND4 | ND4 | CACCTATGACTACCAAAAGCTCATGTAGAAGC | Arévalo et al. (1994) | 94 °C (25 sec), 56 or 60 °C (1 min), 72 °C (2 min) [x25–30] |
| | Leu | CATTACTTTTACTTGGATTTGCACCA | | |
| | S78 | CCTTGGGTGTGATTTTCTCACCT | | |

# Supplementary material I

**Table S1**

Authors: Alejandro Arteaga, Amanda Quezada, Jose Vieira, Juan M. Guayasamin

Data type: excel file.

Explanation note: Locality data for species included in Fig. 2.

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Link: https://doi.org/10.3897/zookeys.1121.86539.suppl1

## Supplementary material 2

**Figure S1**

Authors: Alejandro Arteaga, Amanda Quezada, Jose Vieira, Juan M. Guayasamin

Data type: Image.

Explanation note: Phylogenetic relationships within *Atractus* inferred using a maximum-likelihood approach and derived from analysis of 3,985 bp of DNA (gene fragments 16S, cytb, ND4, c-mos, NT3, and RAG1). Support values on intraspecific branches are not shown for clarity. Voucher numbers for sequences are indicated for each terminal. Black dots indicate clades with bootstrap values from 90–100%. Grey dots indicate values from 70–89%. White dots indicate values from 50–69% (values < 50% not shown). Colored clades correspond to the species' distribution presented in the map of Fig. 2. New or resurrected species are indicated in bold type.

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